**Supporting Materials and Methods**

*Generation and Characterization of* β-*Synuclein Knockout (KO) Mice.* By using genomic clones containing the entire β-synuclein gene, we constructed a targeting vector by standard protocols, as described for the α-synuclein KO (1) except that we made the KO conditional (Fig. 1*A*). Briefly, we flanked the first coding exon (exon 2, encoding residues 1–41) with loxP sites. For positive selection of clones, we inserted a neomycin gene cassette surrounded by frt sites (to remove the neomycin cassette after homologous recombination) into the intron 3' to the floxed exon and, for negative selection, a diphtheria toxin gene 5' to the short arm of the vector. R1 embryonic stem cells (2) were electroporated with the β-synuclein targeting vector, and resistant clones were screened by Southern blot analysis to identify correctly recombined clones (3). One such clone was used to generate mice containing the β-synuclein floxed allele. These mice were crossed to protamine-cre transgenic mice (4) to remove the floxed exon and β-actin Flp transgenic mice (5) to remove the neomycin cassette in the germ line. Genotyping of mutant mice was performed by using the following oligonucleotide primers: SC02105, 5'-AGGACACCACTGGCCCCGAGTCC-3'; SC02106, 5'-GACGCACGTCCGCACGTCCACCC-3'; and SC01114, 5'-TGCCCCTGAAATGCTGCGCC-3', which generated a 320-bp WT band, a 360-bp floxed band, and a 300-bp excised band.

*Western Blotting and Protein Quantitations.* Brains from four adult littermate $\alpha^{-/-}\beta^{+/+}$ and $\alpha^{-/-}\beta^{-/-}$ mice (≈2 months old) were homogenized in PBS with 1 mM PMSF/10 mg/liter leupeptin/1 mg/liter pepstatin A/10 mg/liter aprotinin. Brain homogenates (40 μg per lane) were analyzed by SDS/PAGE and quantitative immunoblotting by using $^{125}$I-labeled secondary antibodies and PhosphorImager detection (Molecular Dynamics), as described for the α-synuclein KO mice (1). GDP-dissociation inhibitor (GDI) and vasolin-containing protein (VCP) were used as internal loading standards for the protein quantitations.

*Electrophysiology.* Transverse hippocampal slices (400 μM thick) were prepared from 3- to 5-week-old mice. Slices were cut on a vibratome (Dosaka, Kyoto) in ice-cold extra cellular solution and kept at room temperature for at least 1.5 h before transfer to the recording chamber. Unless otherwise stated, the external solution contained 119 mM NaCl, 2.5 mM KCl, 3 mM $MgSO_4$, 3 mM $CaCl_2$, 1 mM $NaHPO_4$, 26.2 mM $NaHCO_3$, and 10 mM glucose, saturated with 95% $O_2$/5% $CO_2$ (pH 7.4). A patch-pipette filled with NaCl (1 M) was used to record field excitatory postsynaptic potential (fEPSP) from *Stratum radiatum* of area CA1. Synaptic responses (fEPSPs) were evoked with patch-pipettes (monopolar stimulation) filled with external solution that were positioned in *S. radiatum.* Synaptic depression was examined by using 300 pulses at 14 Hz. Posttetanic potentiation (PTP) was elicited by 30 stimuli at 100 Hz. Long-term potentiation (LTP) was induced in presence of picrotoxin by two stimulus trains of 1 s at 100 Hz with 20-s intervals. To test for the recovery of synaptic responses after depression, synapses were depressed by a burst of high-frequency stimulation (HFS; 100 stimuli at 100 Hz), and fEPSPs were evoked at different time points (0.1–30 s) postburst. All recordings were performed at 25.0 ± 0.1°C. Data were digitized (5 kHz) and analyzed on line by using custom software for IGORPRO (Wavemetrics, Lake Oswego, OR). All data were acquired on littermate offspring from heterozygous matings, analyzed without knowledge of the genotype of the tissue being studied. Genotypes of individual mice were subsequently confirmed by PCR.

*Optical Imaging.* Cortical regions were dissected from 2- to 3-day old mutant mice, and dissociated cultures were prepared by following published protocols (6). Cultures were loaded maximally with FM1-43 (16 μM; Molecular Probes, Eugene, OR) during a 90-s incubation in the hyperkalemic solution 45 mM $K^+$/2 mM $Ca^{2+}$. After loading, cells were washed for 10 min in dye-free solution with nominal $Ca^{2+}$ and imaged. The kinetics of destaining was determined by an application of 10-Hz field stimulation, which was delivered through parallel platinum electrodes that were immersed into the perfusion chamber (30 mA, 1-ms pulses). To determine the total recycling pool size, presynaptic boutons were maximally loaded with FM1-43 and destained by multiple applications of high $K^+$ solution onto the field of interest by gravity (1–2 ml/min). A modified Tyrode

solution used in all experiments contained 150 mM NaCl, 4 mM KCl, 2 mM $MgCl_2$, 10 mM glucose, 10 mM Hepes, and 2 mM $CaCl_2$ (pH 7.4) (≈310 mosmol). High-$K^+$ solutions contained equimolar substitution of KCl for NaCl (90 mM $K^+$). All staining and washing protocols were performed with 10 μM CNQX and 50 μM AP-5 to prevent recurrent activity. Images were obtained by a photometric digital charge-coupled device (CCD) camera (Coolsnap$^{HQ}$, Roper Scientific, Trenton, NJ) during illumination (1 Hz and 40 ms) at 480 ± 20 nm (505 dichroic longpass and 535 ± 25 bandpass) via an optical switch (Sutter Instruments, Novato, CA). Images were acquired and analyzed by using METAFLUOR (Universal Imaging, Downington, PA). Fluorescence imaging data points represent averages of at least two independent experiments. In each experiment, we monitored a total of 500 nerve terminals from several cover slips and averaged all destaining traces for subsequent analysis.

*Light Microscopy.* Gross morphological analysis of mice brains was done by using standard protocols (8). For quantification of dopaminergic neurons, an antibody against tyrosine hydroxylase (TH; Protos Biotech, New York, 1:2,000 dilution) was used to identify the substantia nigra (SN) dopaminergic neurons. Coronal sections, 30 μm thick, were stained with the ABC staining protocol as described (7). The optical fractionator method, an unbiased stereological method was used to count SN TH-containing cell profiles. This method of cell counting that is not affected by either the volume of reference (i.e., SN) or the size of the counted elements (i.e., neurons). This way, 177–398 SN TH cells were counted in each animal ($n$ = 8, 4 male and 2 female per genotype). By using STEREOINVESTIGATOR software (MicroBrightField) cell-profile counting was done in six sections spaced four apart throughout the rostral–caudal extent of the SN. A Leica DMR microscope equipped with a Ludl motorized stage with internal $z$-axis controller was used for these experiments. Each midbrain section was viewed initially at low power (×10 objective), and the SN was outlined by using the set of anatomical landmarks defined previously in our lab. Cells were then identified with a ×100 oil objective (1.3 numerical aperture). By using a random start, the number of TH-stained cells was counted at high power. To avoid double counting, cells were counted only when the nucleus was in clear focus within the counting frame.

*Electron Microscopy.* As described (10), 14-day-old high-density cortical cultures were fixed and embedded for electron microscopy. By using randomized digital electron micrographs, we quantified several synaptic parameters by the following criteria: presynaptic bouton area, the area of a well defined region containing synaptic vesicles; density of synaptic vesicles is the total number of vesicles in a bouton divided by the presynaptic terminal area; active zone length is the presynaptic plasma membrane directly opposing the postsynaptic density; number of docked vesicles is all vesicles within 25 nm of the active zone. All analysis was done blindly, with no prior knowledge of the genotype of the cultures.

*Quantification of Striatal Monoamines.* The dorsal striatum was dissected and sonicated in 0.6 ml of ice-cold 0.1 M perchloric acid to which 6 ng of 3, 4-dihydroxybenzylamine (Sigma) was added as an internal standard. After centrifugation at 8,000 × *g* for 10 min, 20 μl of the clear supernatant was injected into a HPLC reversed-phase column (250 × 4.5 mm, ODS C18, SGE) linked to two coulometric electrochemical detectors by which dopamine, serotonin and metabolites were analyzed as described (8). The mobile phase consisted of a citrate-phosphate buffer (0.04 M citric acid/0.06 M $Na_2HPO_4{\cdot}2H_2O$) containing 0.1 mM EDTA, 0.6 mM 1-heptanesulphonic acid sodium salt, and 10% methanol. Standard curves were established by using regression analysis on the ratios of the peak areas for various concentrations of each compound recorded at the reducing electrode. All experimental values were normalized for protein concentration

*Uptake of $^3$H-Dopamine.* Assays were conducted as described by Ho and Blum (9). In summary, dorsal striata were dissected from WT and mutant mice and used to prepare synaptosomes. Uptake of dopamine was assayed by incubation of synaptosomes with [$^3$H]dopamine [specific activity, 20–40 Ci/mmol (1 Ci = 37 GBq); PerkinElmer], sequential washing, and counting by liquid-scintillation spectrometery. The specificity of uptake was determined by addition of 10 μM mazindol (Research Biochemicals), a high-affinity dopamine uptake inhibitor. Specific high-affinity neuronal dopamine uptake was

calculated as femtomoles of dopamine uptake minus the femtomoles of mazindol uptake per microgram of protein. Values are expressed as percentage of WT.

*Neurotransmitter-Release Assays.* Superfusion assay for neurotransmitter release has been described (10, 11). Dorsal striata were dissected from WT and mutant mice, and used to prepare synaptosomes. These were loaded with 270 nM [$^{3}$H]dopamine (59 Ci/mmol) and 8 μM [$^{14}$C]-γ-aminobutyric acid (GABA) (240 mCi/mmol) for 5 min. Labeled synaptosomes (0.1 ml) were trapped on glass-fiber filters (GF/B; Whatman), overlaid with 50 μl of a 50% Sephadex G-25 slurry, and superfused at 0.8 ml/min with Krebs–bicarbonate buffer at 33°C under continuous aeration with 95% $O_2$/5% $CO_2$ gas mixture. After 10 min of washing, three 1-min fractions were collected to determine baseline release. Neurotransmitter release was stimulated by sequential application of 25 mM KCl and 0.5 M sucrose for 30 s each. The amounts of neurotransmitter secreted into the superfusate and retained in synaptosomes at the end of the experiment were quantified by liquid scintillation counting. Neurotransmitter release, expressed as a fractional release rate, was calculated as the fraction of radioactivity released divided by the amount remaining on the filter at that particular time point.

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